

50 100 Pseudotime (scaled)

Fig. S12. Validation of trajectory inference results using Monocle, SCORPIUS and Slingshot

a Trajectories of cDC2 to migratory DC differentiation by SCORPIUS and Slingshot. b Correlation between cells ordered along their pseudotime using Monocle versus those ordered by SCORPIUS and Slingshot, respectively. **c** Expression of marker genes *BIRC3*, CCR7, CLEC10A and FOS along the DC lineage. d Trajectories of memory B-cells by SCORPIUS and Slingshot. e Correlation between cells ordered along their pseudotime using Monocle versus those ordered by SCORPIUS and Slingshot. **f** Expression of marker genes CD27, IGHA1, IGHD and TCL1A along the memory B-cell lineage. g Trajectories of plasma cell differentiation by SCORPIUS and Slingshot. h Correlation between cells ordered along their pseudotime using Monocle versus those ordered by SCORPIUS and Slingshot. i Expression of marker genes along the pseudotime of the IgA lineage (IGHA1, IGHA2) and IgG lineage (IGHG1, IGHG2). j Branched trajectories of myeloid cells by Slingshot. Trajectories are separated for the CCL18 and MMP9 lineage. k Correlation between cells ordered along their pseudotime using Monocle versus those ordered by SCORPIUS and Slingshot, respectively. I Expression of marker genes along the C5_CCL18 lineage and C6_MMP9 per method. m-n Trajectories of CD8+ T-cells generated by Slingshot(m) and SCORPIUS(n). As SCORPIUS cannot handle branched trajectories, the HAVCR2 and CX3CR1 lineage were analysed separately with SCORPIUS. o Correlation between cells ordered along their pseudotime using Monocle versus those ordered using Slingshot and SCORPIUS, respectively, for the HAVCR2 (C1_CD8_HAVCR2) lineage and the CX3CR1 (C4_CD8_CX3CR1) lineage. p Expression of marker genes CCR7, HAVCR2, PDCD1 along the HAVCR2 lineage and of CCR7, CX3CR1 and *FGFBP2* along the CX3CR1 lineage.